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The impact of post-fire salvage logging on microbial nitrogen cyclers in Mediterranean forest soil

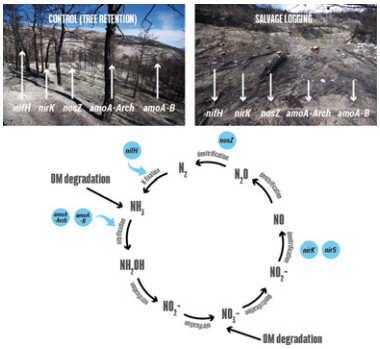
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HIGHLIGHTS

- Salvage logging post-fire affects the properties of Mediterranean forest soil.
- Tree retention post-fire increases pools of *nifH*, *amoA*-B, *amoA*-Arch, *nirK* & *nosZ*.
- OM, avP, N & aggregate stability impact N cycler abundance in forest soil post-fire.
- Microaggregates are hotspots for N cyclers particularly under salvage logging.
- Greater N cycling can support post-fire re-vegetation improving ecosystem recovery.

GRAPHICAL ABSTRACT



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ABSTRACT

Forest fires are a regular occurrence in the Mediterranean basin. High severity fires and post-fire management can affect biological, chemical and physical properties of soil, including the composition and abundance of soil microbial communities. Salvage logging is a post-fire management strategy, which involves the removal of burnt wood from land after a fire. The main objective of this work was to evaluate the impact of post-fire salvage logging and microaggregation on soil microbial communities, specifically on the abundance of nitrogen cyclers and, thus, the potential of the soil for microbial nitrogen cycling. The abundance of nitrogen cyclers was assessed by quantification of microbial nitrogen cycling genes in soil DNA, including *nifH* (involved in nitrogen fixation), *nirS/K* and *nosZ* (involved in denitrification), *amoA*-B and *amoA*-Arch (involved in bacterial and archaeal nitrification, respectively). It was demonstrated that salvage logging reduced bacterial load post-fire when compared to tree retention control and resulted in significant changes to the abundance of functional bacteria involved in nitrogen cycling. Microbial gene pools involved in various stages of the nitrogen cycle were larger in control soil than in soil subjected to post-fire salvage logging and were significantly correlated with organic matter, available phosphorous, nitrogen and aggregate stability. The microaggregate fraction of the soil, which has been associated with greater organic carbon, was shown to be a hotspot for nitrogen cyclers particularly under salvage logging. The impact of post-fire management strategies on soil microbial communities needs to be considered in relation to maintaining ecosystem productivity, resilience and potential impact on climate change.

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1. Introduction

Fires have been a common natural disturbance from late Devonian Period (Schmidt and Noack, 2000). Over the last six decades human

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activity, mainly land use change such as agricultural abandonment, has influenced the pattern, frequency and intensity of fire in the Mediterranean basin (Cerdà and Mataix-Solera, 2009; Pausas and Keeley, 2009). Most currently forested areas in the Mediterranean were previously cultivated but have been abandoned for the last 30–50 years. The lack of management during this transition period from cropping to forest recolonization has led to an accumulation of fuel, resulting in severe fires in some areas (Mataix-Solera and Cerdà, 2009). High severity fires modify soil properties, mainly depending on the peak temperatures, the duration of the fire, soil type and pre-fire conditions (Certini, 2005). Fires affect many biological, chemical and physical properties of soil (Ballard, 2000; Jiménez Esquilin et al., 2008; Yeager et al., 2005), with soil microorganisms being affected even by relatively low temperatures (Neary et al., 1999; Bárcenas-Moreno and Bååth, 2009). Soil microbial communities are also sensitive to changes in other soil properties caused by fire, such as the increase in soil pH from ash accumulation (Mataix-Solera et al., 2009), and volatilization of nitrogen (N) at temperatures around 200 °C (Chandler et al., 1983). Despite a loss of total N by combustion, inorganic N tends to increase as a result of mineralization caused by fire (Rasion et al., 1985; Carballas et al., 1993). This increase however can be very ephemeral because NO_3^- can be easily leached through the soil profile.

Certain post-fire management strategies can have a negative impact on soils, an impact that is potentially more damaging than the fire itself (Mataix-Solera et al., 2015). Salvage logging (SL) is a post-fire management strategy that involves the extraction of burnt wood using heavy machinery, cutting the trunks, and dragging them over the soil surface. Studies on the vegetation dynamic in areas where post-fire SL has been carried out concluded that it had a negative impact on plant recovery (e.g.: González-Ochoa et al., 2003; Martínez-Sánchez et al., 1998; Pausas et al., 2004). However, there is little information on the impact of post-fire SL on soil properties, especially in regards to soil microbial communities. Marañón-Jiménez et al. (2011) observed a decrease in soil respiration after post-fire SL. García-Orenes et al. (2017), observed significant soil degradation in Sierra de Mariola Natural Park, Alcoi, Alicante (E Spain) two years after post-fire SL treatment, which affected the recovery of soil physical, chemical and microbial parameters compared with control areas where burnt trees were retained. Differences between SL treatment and tree-retention controls included reduced soil organic matter (OM) content in topsoil (top 5 cm) to <50%, lower basal soil respiration (BSR), decreased microbial biomass carbon (MBC) and lower aggregate stability (AS). Soil bulk density (BD) increased as a consequence of SL. This was also reflected in the lower density and evenness of vegetation (García-Orenes et al., 2017).

Many soil functions are carried out by soil microbial communities, however, there is little information available on the recovery of microbial functional groups following fires. N cycling is one of most important soil functions carried out by microorganism (Fitter et al., 2005; Wallenstein and Vilgalys, 2005; He et al., 2007). N fixing bacteria reduce N_2 to NH_4^+, producing most of the available N in the biosphere (Brankatschk et al., 2011; Mackelprang et al., 2011). Other microorganisms decompose organic N into NH_4^+ (Zhou et al., 2012) and nitrifying microorganisms oxidize NH_4^+ to NO_2^- and then NO_3^-, the preferred N form for plants (Horz et al., 2004; Fierer et al., 2012). Denitrifying microorganism reduce NO_3^- to NO, N_2O and N_2, returning N to atmosphere and completing the N cycle (Braker et al., 1998; Houlton and Bai, 2009). Therefore, beyond its nutritional value, N cycling is also important in a number of other environmental contexts, for example in controlling the nitrous oxide emission. Our study is adding knowledge on the effects of post-fire management, in particular salvage logging, on N-cycling microbes in forest soils.

The main objectives of this work were to evaluate the impact of post-fire SL on the potential of the soil for microbial N cycling, and relate the soil capacity for N cycling with other parameters of soil health, such as OM, AS, MBC and BSR. Soil potential for N cycling was studied by quantification of genes involved in N cycling in total soil DNA, including *nifH* (involved in N fixation), *nirS/K* and *nosZ* (involved in denitrification) as well as *amoA*-B and *amoA*-Arch (involved in bacterial and archaeal nitrification, respectively). Microbial diversity has recently been shown to be highest in the micro-aggregate fraction of soil (Rabbi et al., 2016), therefore, N cycling potential in the top soil was compared to that in the microaggregate fraction (sieved between 63 and 250 μm). The retention of OM in undisturbed soil, such as under no-tillage cropping systems, stabilizes microaggregates and thus reduces the turnover of macroaggregates (Six et al., 2000). We hypothesize that microaggregates in burnt forest soil subjected to SL, which contains less organic carbon and reduced aggregate stability (García-Orenes et al., 2017), will show decreased microbial N cycling when compared to control areas where trees were retained.

2. Material and methods

2.1. Study site

The study area is located in "Sierra de Mariola Natural Park" in Alcoi, Alicante (E Spain, coordinates: 38°43′59″N, 0°29′16″W). The climate is Mediterranean, characterised by a dry-hot summer and a wet-warm spring, autumn and winter. This area often suffers 3–4 month summer droughts, usually from late June to September. The annual average precipitation is 490 mm, which falls mainly in October–November (maximum rainfall in October, 71 mm). The mean monthly temperature is 14.8 °C (summer: 22.7 °C, winter: 7.9 °C). The forest is composed mainly of *Pinus halepensis* trees, around 40 years of age, with an understory of typical Mediterranean shrubs species, for example *Quercus coccifera*, *Rosmarinus officinalis*, *Thymus vulgaris* and *Brachypodium retusum*. The soil is classified as a Typic Xerorthent (Soil Survey Staff, 2014) developed over marls with a low deep, and is very vulnerable to erosion and degradation processes with a loam soil texture with 45, 39 and 17% of sand, silt and clay, respectively, and 44% of carbonates (García-Orenes et al., 2017).

A forest fire of moderate severity, as defined by Keeley (2009) according to the loss of organic matter above- and belowground, occurred in July 2012 affecting a total of 546 ha. Six months after the forest fire, in February 2013, salvage logging (SL) was carried out in a part of the affected forest. SL consisted of complete extraction of the burned wood using heavy machinery.

2.2. Experimental design and soil sampling

Three plots of 4 m^2 were set up in the burnt forest area where post-fire SL had been carried out (SL) and another three plots in a similarly burnt area nearby where trees were retained, which was used as untreated control (C). In May 2014 (22 months post fire and 15 months after SL treatment) three soil samples (up to 5 cm depth) were collected from the A horizon mineral soil of each plot ($n = 9$ samples per treatment, SL and C), sieved at 2 mm and kept at 4 °C for soil property analyses, and a portion frozen at −20 °C for DNA extraction. A fresh subsample of each sample was sieved through a mesh (63–250 μm) to obtain microaggregates (9 samples per treatment, as above) and a portion was kept at −20 °C for DNA extractions. The samples were denoted: SL-intact core (SL-I), SL-microaggregates (SL-M), C-intact core (C-I) and C-microaggregates (C-M). The properties of the SL and C soils were determined by García-Orenes et al. (2017) and are presented in Table 1.

2.3. DNA extraction and quantitative PCR analysis

DNA was extracted from 0.25 g of each soil sample: SL-I, C-I, SL-M and C-M (nine samples per treatment: three replicate plots and three samples per plot). Total soil DNA extraction was performed using the DNA PowerSoil kit (Mo Bio, Carlsbad, USA) with the following modifications to the manufacturer's instructions: initial vortexing was at 300 rpm for 20 min (STD 3500 Shaker VWR) and DNA was eluted

Table 1
Soil physical-chemical, biological and biochemical properties and plant recovery parameters in May 2014 (García-Orenes et al., 2017).

	Salvage logging	Control[a]
Aggregate stability (%)	71.34 ± 6.04a	84.61 ± 3.93b
Nitrogen Kjeldahl (%)	0.11 ± 0.01a	0.26 ± 0.05b
Available phosphorus (g kg^{-1})	5.94 ± 2.23a	36.5 ± 16.4b
Soil organic matter content (%)	2.62 ± 0.8a	6.61 ± 1.62b
Field capacity (%)	89.6 ± 6.9a	105.2 ± 9.7b
Basal soil respiration (C-CO_2 (μg h^{-1} g^{-1} soil))	1.42 ± 0.37a	2.31 ± 0.48a
Microbial biomass carbon (mg C kg^{-1} soil)	574 ± 72a	1040 ± 81b
Plant richness	9.6 ± 2.5a	12.0 ± 2.0a
Plant evenness	77.4 ± 22.0a	221.5 ± 58.8b
Plant Shannon index H′	0.64 ± 0.10a	0.72 ± 0.03a

Soil samples were from 0 to 5 cm depth. Values in rows sharing the same letter do not differ significantly (one-way ANOVA, $P < 0.05$, $n = 9$).
[a] Control = tree retention.

Table 2
Primer sets used in this study.

Target gene	Primers	Reference
nirS	Cd3aF: GTSAACGTSAAGGARACSGG R3cd: GASTTCGGRTGSGTCTTGA	Throbäck et al. (2004)
nirK	nirk876: ATYGGCGGVCAYGGCGA nirk1040: GCCTCGATCAGRTTRTGGTT	Henry et al. (2004)
nosZ	nosz2F: CGCRACGGCAASAAGGTSMSSGT nosz2R: CAKRTGCAKSGCRTGGCAGAA	Henry et al. (2006)
nifH	MMF2: TNATCACCKCNATCACTTCC MMR1: CGCCGGACKWGACGATGTAG	Pereg and McMillan, unpublished[a,b]
amoA-B	amoa1F: GGGGTTTCTACTGGTGGT amoa2R: CCCCTCKGSAAAGCCTTCTTC	Rotthauwe et al. (1997)
amoA-arch	Arch-AmoAF: STAATGGTCTGGCTTAGACG Arch-AmoAR: GCGGCCATCCATCTGTATGT	Francis et al. (2005)
16S rRNA gene	16 s Forward: TCCTACGGGAGGCAGCAGT 16 s reverse: GGACTACCAGGGTATCTAATCCTGTT	Mohammadi et al. (2003)

Fragments amplified with these set of primers from a variety of soils were cloned into plasmids and the correct size fragments were amplified from 30 transformed *E. coli* colonies for each gene. The fragments cloned were verified as diverse *nifH* sequences.
[a] Based on multiple sequence alignment of available *nifH* sequences from GenBank database to identify conserved regions, and degenerate primers within these regions designed using Primer3 (http://frodo.wi.mit.edu/).
[b] The qPCR *nifH* primers used in this study were designed to overcome issues with previously published primer sets that yielded non-specific products. The qPCR cycle used for these primers is: 95 °C 10 mins; 40 cycles of 15 s 95 °C, 30 s 60 °C, 30 s 72 °C.

with 65 μL elution buffer. DNA yield and purity was measured using a nanodrop (ND-1000 spectrophotometer, NanoDrop Technologies) and the quality of the total DNA (expecting intact DNA of high molecular weight) was further assessed by agarose gel electrophoresis.

Quantitative PCR (qPCR) analyses were used to quantify the total bacterial 16S rRNA gene as well as the N cycling genes in each soil DNA sample. Each sample was assayed in triplicate (three technical replicates of nine independent replicate = 27 per treatment) on a CFX96 Touch Real-Time PCR detection system (Bio-Rad laboratories, CA, USA). Each 25 μL qPCR reaction contained 1× iQ SYBR Green Supermix (Bio-Rad Laboratories, CA, USA), 400 nM each forward and reverse primers, 2.5 μg/μL ultrapure BSA (Invitrogen, CA, USA), 0.5 μL PCR grade DMSO (Sigma-Aldrich, MO, USA), 1 μL of DNA template and RNase/DNase-free water. N cycling genes amplified included 1) *nirS* and *nirK* and *nosZ*, encoding nitrite reductases and nitrous oxide reductase involved in denitrification, 2) *nifH*, encoding the nitrogenase of diazotrophs and 3) archaeal and bacterial *amoA*-Arch and *amoA*-B, respectively, encoding ammonia monooxygenase in nitrifiers (ammonium-oxidizing microbes). The primer sets used and the qPCR cycling conditions for each primer set are shown in Table 2.

PCR products amplified from soil DNA were cloned into pGEM-T using a pGEM-T Easy Vector System II (Promega, WI, USA) to produce standards. Successful cloning and transformation of the target genes was confirmed by sequencing. In all qPCR assays, all samples were amplified in parallel with a triplicate serial dilution (10^1–10^7 gene copies per reaction) of these standards. The efficiencies of qPCR assays were determined by amplification of a serial dilution of soil DNA (5 fold dilution series, from 5 to 0.008 μL DNA per reaction), to give standard curves with efficiencies ranging from 68.9 to 88.7%, with R2 values ranging from 0.988 to 0.997. To assess presence of inhibitors of amplification such as humic and fulvic soil contaminants, the efficiencies and standard curves from amplification of serial dilution of soil DNA were compared to amplification curves and efficiencies from standard plasmids. Soil DNA and standards showed similar efficiencies and standard curve slopes, with no inhibition detected.

Duplicate no-template controls were run for each qPCR assay, which gave null or negligible values. Melt curve analysis and gel electrophoresis were used to confirm amplicon specificity (Data not shown). Gene copy abundance was normalized to gram of dry soil to give results on a biologically significant scale, assuming similar DNA isolation efficiency across samples. Such assumption is appropriate in this study since relative (vs. absolute) quantification was measured.

2.4. Statistical analysis

The adjustment of the data to a normal distribution for all properties analysed was verified with the Kolmogorov-Smirnov test at $P < 0.05$. Measured variables were submitted to a one-way ANOVA, assuming equal variance and the separation of means was carried out according to the average post hoc Tukey test with differences considered significant at $P < 0.05$. Pearson's correlation coefficients (R) were calculated to quantify the linear relationship between parameters.

The relationship between the abundance (copy number g^{-1} soil) of the various genes as well as between gene abundance and soil properties determined by García-Orenes et al. (2017) (Table 1) were analysed by principal component analysis (PCA) with Varimax normalized rotation in order to determine the influence of the post-fire management strategies on the abundance of the N cycling genes. All the results were subjected to a correlation analysis with soil parameters measured using Pearson's rank correlation coefficients. SPSS software (Statistical Program for the Social Sciences 23.0) was used for all statistical analysis.

3. Results

Figs. 1 to 4 show the concentration of total soil DNA, as well as the abundance of the prokaryotic 16S rRNA gene and the six N-cycle genes studied, in intact soil and in the microaggregate fractions of C and SL soil samples.

The average total soil DNA concentrations in SL-I soil samples (9.0 ± 1.6 μg DNA g^{-1} soil) and SL-M (13.8 ± 4.9 μg DNA g^{-1} soil) were 35% lower than in C soil corresponding samples (14.0 ± 2.0 for C-I and 21.0 ± 5.7 μg DNA g^{-1} soil for C-M) (Fig. 1A). Microaggregates had 35% higher concentrations of DNA than intact soil in both SL and C soils (Fig. 1A). These differences in total DNA concentrations were statistically significant. The 16S rRNA gene abundance was also significantly lower in the SL soil (3.4E + 07 and 4.5E + 07 gene copy number g^{-1} soil for SL-I and SL-M, respectively) than in the C soil (1.4E + 08 and 1.7E + 08 gene copy number g^{-1} soil for C-I and C-M, respectively), however, there was no significant difference between 16S rRNA abundance in intact soil and the microaggregate fraction in either the SL or C soils (Fig. 1B).

The abundance of the *nifH* gene, encoding the nitrogenase enzyme in N fixing bacteria, was three fold higher in the intact C-I soil (2.7E + 06 gene copy number g^{-1} soil) than in the intact SL-I soil (1.0E + 06 gene copy number g^{-1} soil) (Fig. 2). However, similar *nifH* copy numbers were found in the microaggregates of SL-M and C-M (2.9E +

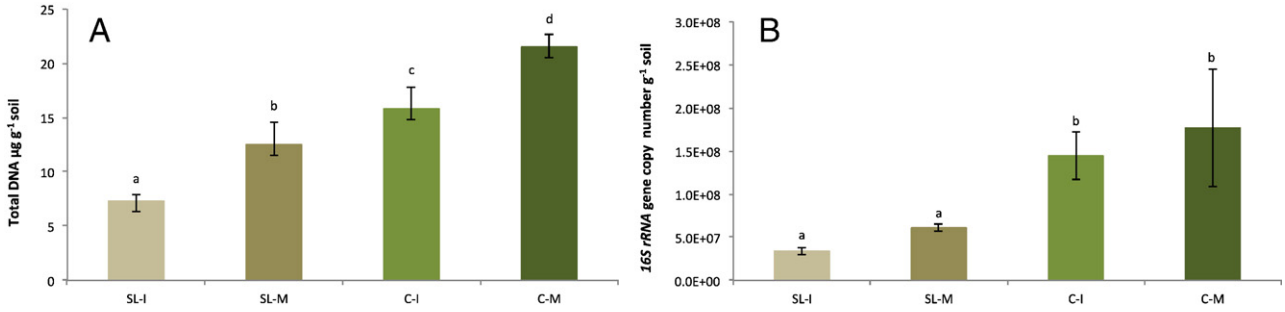


Fig. 1. Abundance of soil total DNA (A) and 16S rRNA gene (B) (mean ± standard deviation) in soils under different treatments analysed by one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 μm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 μm. Different letters above the bars indicate significant differences.

06 and 3.3E + 06 gene copy number g^{-1} soil, respectively) (Fig. 2). While there was a higher abundance of *nifH* in SL-M than in SL-I, the gene abundance in C-M was similar to that in C-I (Fig. 2).

Quantification of genes related to denitrification indicated that *nosZ* was present at higher copy numbers in SL-M (1.6E + 06 gene copy number g^{-1} soil) than in SL-I (6.2E + 05 gene copy number g^{-1} soil) and higher in C-M (3.2E + 06 gene copy number g^{-1} soil) than in C-I (1.9E + 06 gene copy number g^{-1} soil) (Fig. 3A). *nosZ* was also more abundant in C-I than in SL-I, and in C-M than in SL-M. *nirK* was more abundant in the C-I soil (1.7E + 07 gene copy number g^{-1} soil) than in the SL-I soil (8.7E + 06 gene copy number g^{-1} soil) and was also present at greater abundance in the C-M (1.4E + 07 gene copy number g^{-1} soil) than the SL-M (5.7E + 06 gene copy number g^{-1} soil) (Fig. 3B). However, there were no significant differences in copy number between C-I and C-M, or between SL-I and SL-M (Fig. 3B). There were no significant difference in *nirS* copy numbers between any of the samples (Fig. 3C). Copy number per gram of soil were similar in magnitude for *nirK* and *nirS*, and both were approximately a level of magnitude higher than copy number of *nosZ* (Fig. 3).

The distribution of the archaeal and bacterial nitrification *amoA* genes showed similar patterns (Fig. 4). Copy numbers of the *amoA*-Arch (Fig. 4A) and the bacterial *amoA*-B (Fig. 4B) were both higher in C-I (6.4E + 04 and 4.9E + 04 gene copy number g^{-1} soil, respectively) than in SL-I (1E + 04 and 4.1E + 03 gene copy number g^{-1} soil, respectively), as well as higher in C-M (9.7E + 04 and 9.3E + 04 gene copy number g^{-1} soil, respectively) than in SL-M (2.7E + 04 and 2.1E + 04 gene copy number g^{-1} soil, respectively) (Fig. 4). Both genes were also more abundant in C-M than in C-I as well as more abundant in SL-M that in SL-I (Fig. 4). Interestingly, the abundance of the archaeal and the bacterial *amoA* genes had a similar level of magnitude under all treatments (Fig. 4).

Soil properties and plant richness and evenness data determined by García-Orenes et al. (2017) were used to statistically analyse whether there is significant correlation between these factors and the abundance of 16S rRNA gene as well as genes related to the N cycle. Since the previous study only analysed the intact soil (García-Orenes et al., 2017) we used only the data from the gene abundance in intact soil SL-I and C-I in these correlation analyses. A significant positive correlation was previously found between soil OM, N, AP, AS, MBC and BSR (García-Orenes et al., 2017). While total DNA concentration significantly correlated with the soil properties, it only positively correlated with abundance of three genes: *nifH*, *nosZ*, *nirS*, in addition to the 16S rRNA gene (Table 3). All of the soil properties other than MBC were also positively correlated with the abundance of *nifH* (Table 3). The abundance of the 16S rRNA gene, which is present in different bacterial and archaeal species, was positively and significantly correlated with all of the soil properties studied as well as with the abundance of *nirS*, but not significantly correlated with the other genes (Table 3). The abundance of *nosZ* was correlated with soil OM, AP, AS and BSR and the abundance of *amoA*-B with MBC while the abundance of *nirS*, *nirK*, *amoA*-Arch and did not show significant correlation with the soil properties (Table 3).

Correlation analysis on the copy numbers of different genes involved in the N cycle showed that the abundance of soil *nosZ, nirS* and *amoA*-Arch were significantly correlated (Table 3). The abundance of *nifH* was correlated to that of *nosZ*, but *nirK* and *amoA*-B showed no significant correlation with any of the other N cycling genes (Table 3).

PCA was performed to evaluate the influence of the post-fire management strategy on the abundance of the 16S rRNA gene and N cycling genes in both intact soil and in the microaggregate fraction of soils (Fig. 5). The first two components explained 90% of the total variation with eigenvalues >1.0. The first component PC1 explained 70% of the variation and separated C from SL, with *nirS*, *nifH* and *amoA*-Arch having

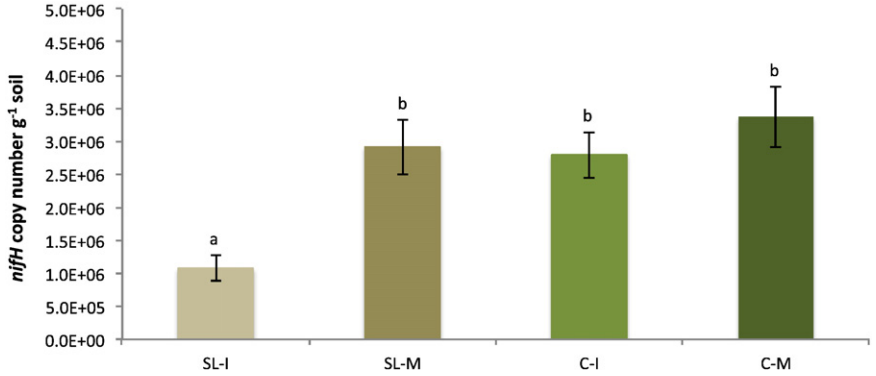


Fig. 2. Abundance of *nifH* gene (mean ± standard deviation) in soil with different treatment after one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 μm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 μm. Different letters above the bars indicate significant differences.

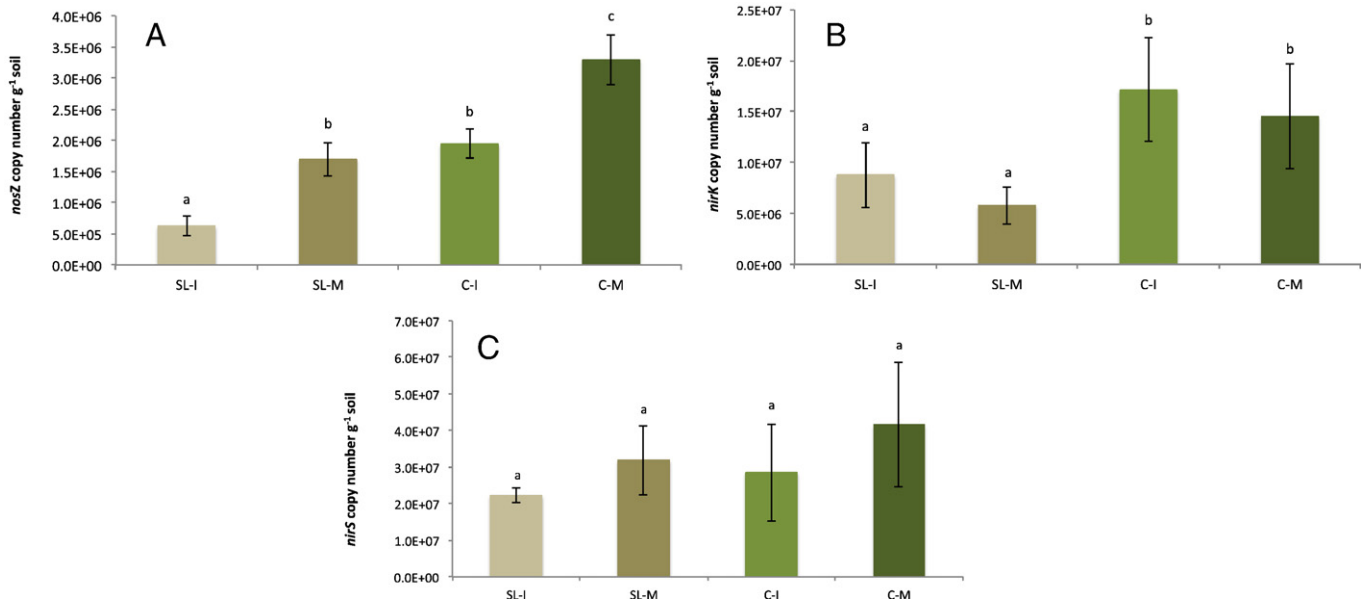


Fig. 3. Abundance of *nosZ* gene (A), *nirK* gene (B) and *nirS* gene (C) (mean ± standard deviation) in soil with different treatment after one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 μm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 μm. Different letters above the bars indicate significant differences.

loading values of 0.93, 0.91 and 0.89, respectively. The second component PC2 explains 20% of the variance and separated the intact soil of each treatment from its microaggregate fraction with *nosZ* and *16S rRNA* gene having loading values of 0.70 and 0.68, respectively.

Fig. 6 shows the result of a PCA, which included all of the soil properties studied (Table 1), total DNA and the abundance of 16S rRNA gene and genes involved in N cycling from intact soil samples. The two first components have explained 88.9% of the variance with eigenvalues >1.0. The first component PC1 explained 79.7% of the variation and separated Soil C from Soil SL, with OM, N, AS, MBC and BSR having loading values of 0.96, 0.95, 0.93, 0.85 and 0.79, respectively.

4. Discussion

Our study enhances the knowledge on the effect of post-fire forest management on the abundance of soil bacteria, in particular those involved in N cycling. Our results show a decline in abundance of the 16S rRNA gene after salvage logging, suggesting that, while tree retention allows the recovery of soil bacterial communities in burnt forest soil, SL reduces the ecosystem resilience and recovery of bacterial communities. This is in agreement with previous finding of Dahlberg et al. (2001) and Twieg et al. (2007), who found a decline in the abundance of microorganism after salvage logging, and Holden et al. (2016), who demonstrated that forest clearing has a negative impact on soil microbes. Tree management by partial harvesting appears to have less impact on microbial biomass in soils with low pH and high carbon content (Gömöryová et al., 2017). García-Orenes et al. (2017) found that SL resulted in slower recovery of MBC and BSR in Sierra de Mariola. High correlations of OM content, N and available P with bacterial abundance in neutral soil (pH approx. 8.0 for all samples in C and SL) of Sierra de Mariola, suggest that these soil parameters could explain the lower abundance of bacterial 16S rRNA gene under SL. Nevertheless, other soil properties, including physical parameters, such as soil compaction, should not be ignored. Timber harvesting using heavy machinery has been shown to have a significant impact on soil microbial communities in Northern coniferous forests (Hartmann et al., 2012) and controlled fundamental experiments showed that forest soil compaction was associated with a persistent change in soil microbiota, with sandy soils being more resistant to the effects of compaction than clayey soils (Hartmann et al., 2014). Indeed, the bulk density of the relatively sandy soil (45%) of Sierra de Mariola measured in May 2014 was higher under SL (0.8 + 0.05 g cm^{3-1}) than under control (C) conditions (0.72 + 0.05 g cm^{3-1}) (García-Orenes et al., 2017), suggesting that compaction in addition to tree removal could possibly be the cause of the decrease in soil bacterial abundance in SL soil. Compaction affects moisture and oxygen levels in the soil, with anaerobic bacteria being significantly associated with compacted forest soils (Hartmann et al., 2014).

Our results clearly demonstrate the importance of microaggregation, in particular in the disturbed SL soil. Rabbi et al. (2016) observed higher diversity of bacteria in soil microaggregates than in macroaggregates,

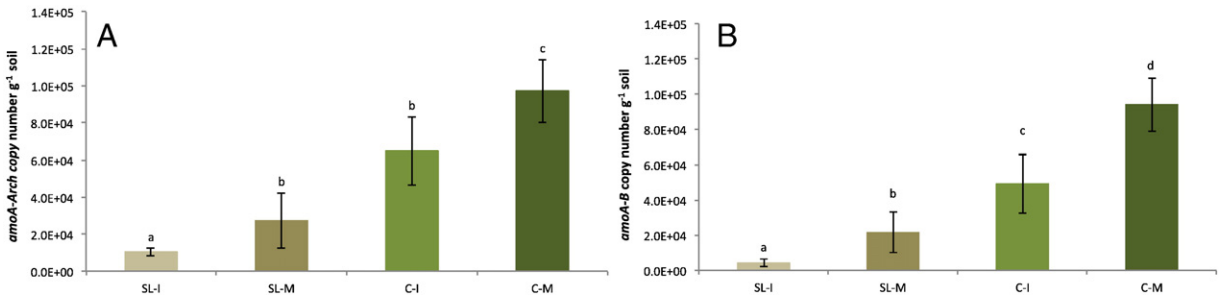


Fig. 4. Abundance of *amoA*-Arch gene (A) and *amoA*-B (B) (mean ± standard deviation) in soil with different treatment after one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 μm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 μm. Different letters above the bars indicate significant differences.

Table 3
Correlation coefficients (*r* values) for relationships between the different physico-chemical soil properties determined and genes of soil ($n = 18$).

Parameter[a]	OM	N	AP	AS	MBC	BSR	*nifH*	*nosZ*	*nirS*	*nirK*	*amoA*-Arch	*amoA*-B	16S rDNA	Total DNA
OM	1	0.95**	0.91**	0.77**	0.75**	0.78**	0.70**	0.47*	ns	ns	ns	ns	0.76**	0.84**
N		1	0.88**	0.81**	0.80**	0.70**	0.68**	ns	ns	ns	ns	ns	0.79*	0.83**
AP			1	0.78**	0.66**	0.75**	0.74**	0.49*	ns	ns	ns	ns	0.63**	0.70**
AS				1	0.69**	0.54*	0.64**	0.56*	ns	ns	ns	ns	0.76**	0.63**
MBC					1	0.51*	ns	ns	ns	ns	ns	0.62**	0.72**	0.59**
BSR						1	0.53*	0.66**	ns	ns	ns	ns	0.53*	0.71**
nifH							1	0.67**	ns	ns	ns	ns	ns	0.67**
nosZ								1	ns	ns	0.56*	ns	ns	0.59*
nirS									1	ns	0.56*	ns	0.65**	0.66**
nirK										1	ns	ns	ns	ns
amoA-Arch											1	ns	ns	ns
amoA-B												1	ns	ns
16S rDNA													1	0.77**
Total DNA														1

ns: not significant. 16S rDNA: 16S rRNA gene. The results were confirmed using a non-linear method (Spearman) and similar values were obtained.
[a] OM: soil organic matter; N: Kjeldahl nitrogen; AP: available phosphorus; AS: aggregates stability; MBC: microbial biomass carbon; BSR: basal soil respiration.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

which was highly correlated with organic carbon in pasture, crop and woodland soils. The significant correlation between 16S rRNA gene abundance, OM and AS, suggests that the combination of OM and higher degree of aggregation in Soil C (control soil) support greater bacterial abundance than in forest soil that has undergone SL.

The quantification and characterization of N cycling communities, including N-fixation, denitrification and nitrification, can help assess ecosystem functioning in natural and agricultural systems (Kowalchuk and Stephen, 2001; Philippot, 2002), and provide information for predicting and mitigating greenhouse gas emissions (reviewed by Levy-Booth et al., 2014). Burning and logging can affect the concentrations of OM and N in soils (Grogan et al., 2000; Neary et al., 1999; Wan et al., 2001; García-Orenes et al., 2017) and the N-cycling microbial communities (Reich et al., 2001; Shaffer et al., 2000; Walley et al., 1996). The higher abundance of bacteria and archaea, especially those involved in N cycling, in control soil without post-fire tree removal than after SL is especially important in burned soil since improved N cycling could increase N pools available for re-vegetation following fire. This is in agreement with the higher plant richness and evenness in control soil than in the soil following post-fire SL.

4.1. Effect of post-fire treatment on nitrogen fixation genes abundance in soil

Fire and logging both have long-term impacts on N-cycling bacteria (Goodale and Aber, 2001; Kennedy and Egger, 2010). In agreement with Shaffer et al. (2000), who concluded that logging removes a unique *nifH* gene pool from the soil in Douglas-fir forests, probably as a consequence of loss of organic material, we have shown that SL has decreased the abundance of *nifH* in fire affected soil when compared to control areas without tree removal.

Similarly to Kennedy and Egger (2010), Levy-Booth and Winder (2010) and Morales et al. (2010) we observed greater *nifH* abundance significantly associated with higher organic carbon (in our study OM), in addition to higher P and AS. Since N fixation requires large amounts of adenosine triphosphate (ATP) and reducing equivalents to fuel the process, heterotrophic diazotrophs are highly dependent on availability of carbon (Chan et al., 1997) and P, explaining the lower abundance of *nifH* in OM- and P-poor SL soil compared to C forest soil. The gene *nifH* gene was observed at greater abundance in microaggregates in the

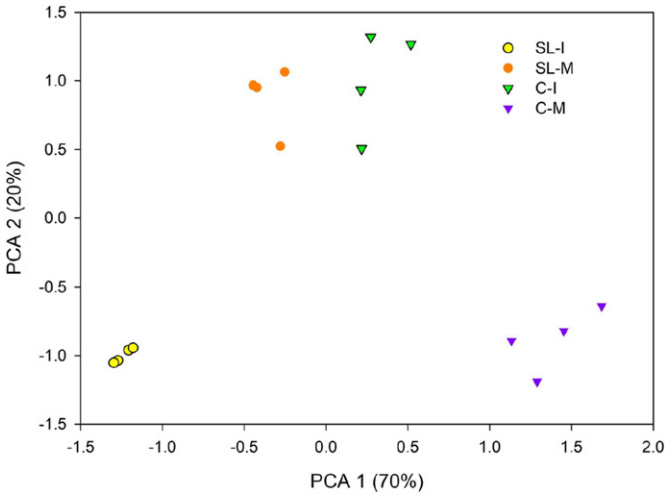


Fig. 5. Principal component analysis performed on the abundance of different soil N cycling genes studied (*nifH*, *nirS*, *niK*, *nosZ*, *amoA*-Arch and *amoA*-B) and 16 s rRNA gene. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 μm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 μm. Different letters above the bars indicate significant differences.

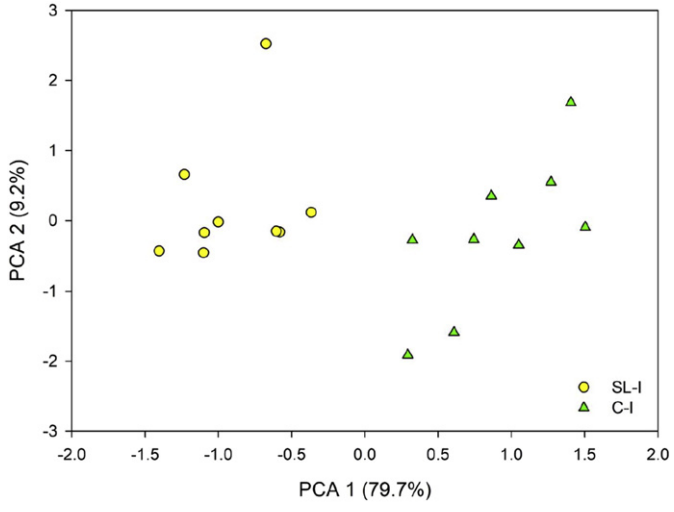


Fig. 6. Principal component analysis performed on N cycling genes and other soil properties. Gene abundance included *nifH*, *nirS*, *niK*, *nosZ*, *amoA*-Arch, *amoA*-B and 16 s rRNA gene, and soil properties included SOC: soil organic carbon; N: Kjeldahl nitrogen; AP: available phosphorus; AS: aggregates stability; MBC: microbial biomass carbon; BSR: basal soil respiration. SL: soil under salvage logging treatment; C: control soil with tree retention.

degraded SL soil, but not in the control soil. The control soil has higher OM and AS (García-Orenes et al., 2017) possibly supporting greater *nifH* abundance throughout the intact top-soil, while in the SL soil the carbon loaded microaggregate fraction (Rabbi et al., 2016) possibly provides an advantageous niche for anaerobic N fixing bacteria. As suggested by Kennedy and Egger (2010), other types of N fixing microbes, such as photosynthetic cyanobacteria, may actually benefit from the loss of the tree canopy in SL soils since it allows greater light and increased soil temperatures (Ballard, 2000).

4.2. Effect of post-fire treatment on denitrification genes abundance in soil

The nitrite reductase encoding genes, *nirK* and *nirS*, are often used as molecular markers of the denitrification process (Braker et al., 1998). In various natural environments, in particular aquatic environments, *nirS* has been found to dominate over *nirK* (Bothe et al., 2000; Liu et al., 2010; Throbäck et al., 2004; Oakley et al., 2007; Deslippe et al., 2014), however, *nirK* encoding the copper-containing nitrite reductase has been found to prevail in aerobic, oxygen-rich, environments (Desnues et al., 2007). In our study *nirS* and *nirK* were present in the soil at the same order of magnitude, however they showed different responses to SL. The abundance of these two genes is influenced by a range of factors, including soil moisture and temperature (Szukics et al., 2010; Rasche et al., 2011), total N concentration (Kandeler et al., 2009; Levy-Booth and Winder, 2010), concentration of available P, and soil OM (Petersen et al., 2012). García-Orenes et al. (2017) showed a decrease in total N, available P and soil OM in the SL managed soils. Although such reductions can explain a decrease in the abundance of the *nirK* and *nosZ* genes in post-fire SL soil, there was no correlation found between these parameters and *nirK* in our study. There were also differences in the distribution of these genes in the soil, with *nosZ* abundance being higher in the microaggregate fraction in both SL and control soils while *nirK* showed similar abundance in the intact soil and the microaggregate fraction. In agreement with Zhang et al. (2013), who reported mixed responses of denitrification genes, while *nosZ* and *nirK* showed a decrease in SL soils compared with C soil, the abundance of *nirS* was similar under all treatments.

The size of the denitrifying community may also indicate the greenhouse gas emission potential and aboveground net primary productivity of soils (Morales et al., 2010). The detection of *nosZ* in the soil suggests the presence of denitrifiers with the ability to reduce N_2O to N_2 (Miller et al., 2008). Post-fire management, as our study showed, has the potential to greatly modify the size and structure of denitrifying bacterial communities in the soil, and could therefore have a great influence on gas emissions. This fact must be considered in current large-scale models for climate change and other global phenomena (Morales et al., 2010).

4.3. Effect of post-fire treatment on nitrification genes abundance in soil

The *amoA* gene has been used in molecular studies of AOB (ammonia-oxidizing bacteria) and AOA (ammonia-oxidizing archaea) communities (Norton et al., 2002). It has been reported that plant species, temperature, water content, C:N ratio and soil total N are strongly linked with the AOA and AOB community structure (Boyle-Yarwood et al., 2008; Rooney et al., 2010; Szukics et al., 2010; Rasche et al., 2011; Zeglin et al., 2011; Szukics et al., 2012). In our study post-fire SL significantly reduced the abundance of both archaeal and bacterial *amoA* (ammonia oxidisers) and both AOA and AOB *amoA* genes were found in higher concentrations in the microaggregate fraction than in the intact soil. Archaeal *amoA* genes have been found in diverse environments, for example oceans (Francis et al., 2007), and soils (Leininger et al., 2006; Di et al., 2010). AOB-*amoA* genes have been found to dominate over AOA-*amoA* in some soils (Di et al., 2010), while AOA-*amoA* is more abundant in other soils (Leininger et al., 2006). Interestingly, despite the requirements of less oxygen by AOA ammonia oxidisers (Levy-Booth et al., 2014 and references within), in our study they were found to be present in similar numbers, both concentrated in the soil microaggregates, and they were similarly affected by SL. The post-fire management, which has affected soil properties, especially plant species, water content by structure modification (field capacity), and C (carbon) and N content in the soil (García-Orenes et al., 2017), also influenced the abundance of archaeal and bacterial *amoA*. The AOB community responds to changes in pH and ammonia availability, both commonly increasing after forest fires, with pH increase being associated with enhanced bacterial *amoA* gene clusters (Levy-Booth et al., 2014). Both AOA and AOB are relatively slow growing (Prosser and Nicol, 2008), which could explain the lower overall abundance compared to other N-cyclers found in this and other work.

5. Conclusions

Functional gene assessment can determine the relationship between microbial communities and ecosystem functions and can be a useful tool to select the optimal post-fire forest management. We have demonstrated in this work that post-fire management strategy, SL or C, significantly impacts on the community structure of N cycling microbes in forest soil. Soil microbes were present at higher abundance in C soil, where trees were retained post-fire, and in soil microaggregates. Soil properties that were affected by SL, in particular a reduction in OM, available P, N and AS, were accompanied by changes in microbial soil communities, including functional bacteria involved in N cycling.

Microbial gene pools involved in the N cycle, including N fixation, bacterial and archaeal nitrification and denitrification, recovered faster in control soil without post-fire tree removal than after SL. This fact is especially important in burned soil because improved N cycling could increase N pools available for re-vegetation following fire. This is supported by the control soil having higher plant richness and evenness than the soil following post-fire SL.

The importance of the microaggregate fraction for soil function is highlighted in this work with the microaggregates proving to be an important hot-spot for bacterial abundance, especially in the disturbed SL soil. The control soil with its organic matter and aggregate stability supported bacteria and N cyclers in the bulk soil, not only in the microaggregates.

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